

AUTONOMIC INNERVATION OF THE RAT
URINARY BLADDER

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This thesis is based on the following papers which in the text will be referred to by their Arabic numerals:

1. Degeneration activity in the rat urinary bladder. *Acta physiologica scandinavica* 1973. 87. 223—227.
2. Action of drugs on the innervated and denervated urinary bladder of the rat. *Acta physiologica scandinavica* 1974. 91. 289—297.
3. Inhibitory β -adrenoceptors in the urinary bladder of the rat. *Life Sciences* 1974. 15. 273—280.
4. Atropine sensitivity of the rat urinary bladder during nerve degeneration. *Acta physiologica scandinavica* 1975. In press.
5. Adrenergic and cholinergic innervation of the rat urinary bladder. *Acta physiologica scandinavica* 1975. In press. (Together with P. Alm.)
6. Stimulation of autonomic nerves to the urinary bladder of the rat. Submitted for publication in *Experientia* 1975.
7. Stimulation of adrenergic nerve fibres to the urinary bladder of the rat. *Acta physiologica scandinavica* 1975. In press.

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INTRODUCTION

According to the classical concept the urinary bladder is innervated by both parasympathetic and sympathetic nerves and constitutes an example of antagonism between the two divisions of the autonomic nervous system. Dual innervation of the bladder was first demonstrated by Giannuzzi (1863) in Claude Bernard's laboratory showing that impulses to contraction of the detrusor muscle can reach the dog bladder either through sacral spinal roots in the (parasympathetic) pelvic nerves or through lumbar roots in the (sympathetic) hypogastric nerves.

Sympathetic inhibition causing relaxation of the detrusor has been demonstrated in the dog (von Zeissl, 1893; Griffiths, 1895), in the cat (Elliott, 1907) and in the dog and cat (Kuntz and Saccomanno, 1944). A contractile response to hypogastric nerve stimulation has been shown in the cat and monkey (Sherrington, 1892), in the dog, cat and rabbit (Langley and Anderson, 1895) and in the guinea-pig (Mantegazza and Naimzada, 1967). Debaisieux (1912) found no antagonism between the parasympathetic and sympathetic nerves in the dog, cat and rabbit but only synergistic contractions when stimulating the pelvic and hypogastric nerves simultaneously. Edvardsen (1968), however, demonstrated antagonistic effects of concomitant pelvic and hypogastric nerve stimulation in the cat. In the rat Vanov (1965) found no effect of sympathetic nerve stimulation on the bladder.

It is generally accepted that stimulation of the parasympathetic pelvic nerves produces contraction of the detrusor muscle, but the problem of the nature of the parasympathetic postganglionic neurone in the urinary bladder has remained unsolved (see Ambache, 1955 and Taira, 1972). The excitatory effect of exogenous acetylcholine on the bladder is antagonized by atropine, while the response to pelvic nerve stimulation is almost completely atropine resistant (Langley and Anderson, 1895). Because of this anomaly it has been proposed that the parasympathetic motor nerves of the bladder are non-cholinergic in the dog (Henderson, 1923; Henderson and Roepke, 1934), in the guinea-pig, cat and rabbit (Ambache and Zar, 1970) and in

the guinea-pig and rat (Dumsday, 1971; Burnstock, Dumsday and Smythe, 1972). It has been suggested that these nerves instead release a purine nucleotide *e.g.* adenosine triphosphate (ATP) as transmitter and the term "purinergic nerves" has been proposed for such nerves (Burnstock, 1971).

The purpose of the present investigation was to study the nature and function of the different autonomic nerves to the urinary bladder by means of injection of autonomic drugs, denervation experiments, histochemistry and nerve stimulation. The male rat was used because the rat bladder in contrast to other species contains no intramural ganglion cells (Carpenter and Rubin, 1967; Chesher, 1967; 5) and the pelvic plexa form distinct ganglia in the male rat but not in the female (Langworthy, 1965). Thus it is possible to denervate the urinary bladder or to stimulate the parasympathetic nerves postganglionically, which is difficult in most other organs, since the postganglionic neurone in the parasympathetic system usually is situated within the effector organ.

CHAPTER I

METHODOLOGY

Intravesical pressure recording

The intravesical pressure was recorded *in situ* by way of a glass cannula inserted into the bladder through an incision in the exposed urethra (1). The ureters were ligated and the glass cannula was fixed by a ligature around the bladder neck immediately below the ureters in order to avoid influences on the pressure recordings from the sphincter region at the base of the bladder (see Figure). Care was taken not to damage the nerves reaching the detrusor muscle beside and above the ureters. The bladder was filled with the same volume of physiological saline (0.25 ml) in all experiments since the pressure developed during a response varies in proportion to the volume; the tension in the wall is directly proportional to the radius and the pressure according to Laplace's law. The resting pressure was about 10 mm Hg in all experiments.

The rats were anaesthetized with chloralose (100 mg/kg) given through a cannula in a femoral vein after induction with ether. The drugs used were injected through the cannula in the femoral vein.

Denervation procedures

To make a postganglionic sympathetic denervation the hypogastric nerves were cut distal to the hypogastric ganglia at the bifurcation of the aorta. The sympathetic denervation was probably not complete since adrenergic nerve fibres have been detected histochemically in the pelvic nerves of the rat (5). Parasympathetic decentralization was performed by section of the pelvic nerves proximal to the pelvic ganglia on the lateral surface of the prostate gland. At this procedure some postganglionic cholinergic and adrenergic fibres were also cut (5). The bladder was postganglionically denervated by extirpation of the pelvic ganglia. This procedure results in sympathetic as well as parasympathetic denervation since the postganglionic fibres of the hypogastric nerves pass through the pelvic ganglia (see Figure).

The operations were made aseptically using ether anaesthesia. In rats with divided parasympathetic nerves the bladder had to be emptied daily by manual pressure in ether anaesthesia. Despite this treatment these bladders always contained some residual urine and increased in weight. The animals were given a sulfonamide to avoid bacterial infection.

Histochemistry

For demonstration of adrenergic nerves the fluorescence method of Falck and Hillarp was used (5). In order to differentiate long and short adrenergic neurones some rats were pretreated with reserpine or 6-hydroxydopamine. Acetylcholinesterase activity was demonstrated using the copper thiocholine method of Koelle and Friedenwald as modified by Holmstedt (5).

Nerve stimulation

For electrical stimulation the hypogastric nerves were cut distal to the hypogastric ganglia and the distal ends were stimulated jointly, using a bipolar electrode. Each pelvic nerve was stimulated after section proximal or distal to the pelvic ganglion. Before postganglionic pelvic nerve stimulation the hypogastric nerves were cut and allowed to degenerate in order to avoid stimulating the fibres from the hypogastric nerves which pass through the pelvic ganglia. Grass stimulators supplied with stimulus isolation units, giving rectangular pulses with a duration of 2 ms as single shocks or at frequencies between 0.1 and 200 Hz and of supramaximal intensity (3–10 V) were used. Unless stated otherwise the nerves were stimulated for 15 s, *i.e.* for a period approximately corresponding to that of a spontaneous micturition contraction (Edvardsen, 1968).

Statistical analysis

For statistical evaluation of the data Student's t-test for paired or unpaired samples was used. The 0.05 level of probability was accepted as significant.

CHAPTER II

ACTION OF DRUGS

Parasympathomimetic drugs

Methacholine and acetylcholine caused contraction of the detrusor muscle increasing the intravesical pressure; the responses were totally abolished by previous injection of atropine (2). The excitatory effect of parasympathomimetic agents on the detrusor muscle was also found by Edmunds and Roth (1920) in the cat, by Carpenter (1963) in the rat *in vitro* and by Vanov (1965) in the rat *in situ*.

Sympathomimetic drugs

Adrenaline and phenylephrine elicited contraction of the detrusor, while isoprenaline caused relaxation. The response to noradrenaline varied but was most often relaxation (2). Elliott (1905) found no response to adrenaline in the rat bladder by ocular inspection and Vanov (1965) got no effect of either adrenaline or noradrenaline using pithed rats and recording the responses of the bladder as contractions in the axis from the urethra to the vertex, instead of changes in the intravesical pressure.

The responses in rats to the catecholamines can be expressed in terms of α - and β -adrenoceptors (Ahlquist, 1948), as is the case in the urinary bladder of cats and rabbits (Edvardsen and Setekleiv, 1968; Sjöstrand, Sjögren and Schmitterlöw, 1972) and dogs (Dhasmana, Gupta and Bhargava, 1970). The β -stimulating agent isoprenaline imitated the inhibitory response to noradrenaline and after β -receptor blockade using propranolol the response to noradrenaline was always a contraction (2). The excitatory response to adrenaline was mimicked by the α -stimulating agent phenylephrine and after α -receptor blockade with dihydroergotamine adrenaline caused relaxation of the bladder. Adrenaline exerted its effect mainly on the excitatory α -receptors, but the contraction caused by adrenaline was increased after β -receptor blockade, indicating that normally the inhibitory β -receptors were also stimulated. Thus the presence of both excitatory α -receptors and inhibitory β -receptors in the detrusor muscle of the rat was

shown (2). The detrusor muscle of the human urinary bladder is supplied with adrenergic as well as cholinergic nerve fibres (Mobley, El-Badawi, McDonald and Schenk, 1966) and contains both α - and β -adrenoceptors (Awad, Bruce, Carro-Ciampi, Downie, Lin and Marks, 1974).

The existence of two types of β -adrenoceptors has been suggested (Lands, Luduena and Buzzo, 1967), viz. β_1 , in the heart and small intestine, and β_2 , in the bronchi, blood vessels and uterus. The β_2 -adrenoceptor stimulating agents terbutaline and salbutamol caused relaxation of the detrusor muscle decreasing the intravesical pressure (3). The β_1 -receptor blocking compounds practolol and H ⁹³/₂₆, acting selectively on excitatory cardiac receptors, did not affect the inhibitory bladder response to noradrenaline (unpublished observation), isoprenaline, terbutaline or salbutamol, which was, however, completely abolished by propranolol or the β_2 -blocking agent H ³⁵/₂₅ (3). It may thus be concluded that the β -adrenoceptors of the rat urinary bladder belong to the type of inhibitory receptors classified as β_2 -receptors in other organs. The nature of the β -receptors in the urinary bladder has not yet been investigated in other species.

DENERVATION EXPERIMENTS

Acute effects of denervation

Section of the pelvic or hypogastric nerves caused no acute changes in bladder tone or resting activity indicating that the detrusor muscle in the deeply anaesthetized rat is not under parasympathetic or sympathetic tone at the bladder volume studied. In Elliott's experiments (1907) in the cat section of both pelvic nerves lowered the tone of the muscle, leaving, however, its spontaneous rhythm.

Section of one pelvic nerve did not change the bladder response to electrical stimulation of the opposite pelvic nerve (6). In the dog the response to stimulation of the distal cut end of one pelvic nerve was reduced after section of the opposite pelvic nerve (Diokno, Davis and Lapidès, 1973). It was suggested that the reflex arc of the side with intact nerve was activated by the distension of that side of the bladder caused by contraction of the stimulated half of the detrusor. Section of the hypogastric nerves did not affect the responses to pelvic nerve stimulation or *vice versa* (6), which was also found by Ingersoll, Jones and Hegre (1957) in the dog and by Edvardsen (1968) in the cat.

Early effect of denervation

During the first few days after section of postganglionic autonomic nerves activity in the effector organ has been described. The activity is due to a leakage of transmitter from the degenerating nerve terminals (see Emmelin and Trendelenburg, 1972). During the second and third day after postganglionic denervation periods of increased activity were found in urinary bladders which had been sensitized by earlier parasympathetic decentralization or sympathetic denervation (1). In the parotid gland, which also belongs to the few structures in which the postganglionic parasympathetic fibres can be cut, the degeneration secretion starts and ceases earlier than the activity in the urinary bladder, but it has about the same duration and may also appear in periods (Emmelin and Strömblad, 1958). It seems

reasonable to conclude that the increased activity in the urinary bladder after postganglionic parasympathetic denervation is a degeneration activity.

Injection of eserine produced an increase of the degeneration activity (1). Bladder activity produced by electrical stimulation of the vesical nerves is also potentiated by anticholinesterases (Carpenter, 1963; Vanov, 1965; Chesher, 1970; 5; 6). While the detrusor response to stimulation of the parasympathetic nerves is atropine resistant (Langley and Anderson, 1895), the vesical response to the transmitter supposed to leak from the degenerating nerve endings could be totally abolished by atropine or the parasympatholytic agent Hoechst 9980, suggesting that acetylcholine nevertheless acts as transmitter (1).

Since some adrenergic nerve fibres could be demonstrated histochemically in the pelvic nerve (5), transmitter might be expected to leak from these fibres too after section of the nerve. However, no degeneration activity could be detected after atropine, which may be explained by the sparse adrenergic innervation of the detrusor muscle (El-Badawi and Schenk, 1966; 5).

To explain the atropine resistance Hukovič, Rand and Vanov (1965) suggest that the acetylcholine released by nerve impulses may reach high concentration locally, sufficient to surmount the blockade of the receptors by atropine. It is reasonable to assume that the amount of transmitter leaking from the degenerating nerve endings is smaller than that liberated by electrical stimulation of the intact nerve as is the case in other organs (see Emmelin and Trendelenburg, 1972). Dale and Gaddum (1930) suggest that acetylcholine is liberated by the nerve endings in such an intimate relationship to the receptor that atropine cannot prevent its access. A similar theory is proposed for the urinary bladder of the dog and rabbit (Ursillo and Clark, 1956) and rat (Chesher, 1970). The atropine sensitivity of the degeneration activity found in paper 1 may be consistent with these theories provided that the distance between nerve terminal and receptor increases during the second and third day after denervation as is found in somatomotor neuromuscular junctions (Reger, 1959). Whether this is the case in the urinary bladder is, however, not known.

Late effects of denervation

Disappearance of nerve fibres. The detrusor muscle of the rat has a sparse adrenergic innervation and a rich supply of acetylcholinesterase (AChE)-positive nerves (El-Badawi and Schenk, 1966; 5). The adrenergic innervation was found to originate from long adrenergic neurones and neither

adrenergic nor AChE-positive ganglion cells could be demonstrated within the bladder wall despite serial sectioning (5).

Two weeks after postganglionic sympathetic denervation no detectable change of adrenergic or AChE-positive nerves was found in the bladder, while parasympathetic decentralization or denervation produced a total disappearance of adrenergic nerves (5). The AChE-positive nerves were appreciably reduced in number after parasympathetic decentralization and not detectable after postganglionic denervation (5).

It may be concluded that the urinary bladder of the rat is mainly supplied by adrenergic nerves *via* sacral nerve trunks and not *via* the hypogastric nerves. Bundles of adrenergic nerve fibres were demonstrated in the pelvic nerves as well as in the hypogastric nerves (5). This is in accordance with previous findings in cats (Hamberger and Norberg, 1965; Sundin and Dahlström, 1973) but is contrary to findings in guinea-pigs (Wakade and Kirpekar, 1972). The presence of adrenergic fibres innervating the bladder in both the hypogastric and pelvic nerves is supported by the histochemical demonstration of adrenergic fibres in both nerves and by the stimulation experiments to be described in chapter IV. The AChE-positive fibres probably pass to the bladder mainly *via* the pelvic nerves having their cellbodies outside the bladder wall in the pelvic ganglia and to some extent proximal to the pelvic ganglia (see Figure). The number of cholinergic fibres in the bladder wall originating from the hypogastric nerves might be expected to be much smaller than that from the pelvic nerves, and this may explain why no reduction of AChE-positive nerves could be detected at sympathetic denervation.

Supersensitivity. Increased sensitivity to injected methacholine and acetylcholine was found two weeks after parasympathetic decentralization and denervation and after sympathetic denervation (2). Development of supersensitivity in the parasympathetically decentralized bladder has earlier not been studied in experimental animals (see Taira, 1972). Carpenter and Rand (1965) found no increase of the sensitivity to parasympathomimetic agents in the rat bladder after denervation, which may be explained by the different methods used. They stated that section of the pelvic nerves caused lesions of the blood supply to the bladder, and instead of emptying the bladder daily by manual pressure, they placed a ligature around the bladder neck above the ureters. Unilateral denervation does not cause any supersensitivity to parasympathomimetic drugs, possibly because the axons from either side distribute bilaterally in the rat detrusor muscle (Chapter IV).

Either parasympathetic or sympathetic denervation caused supersensi-

tivity to both parasympathomimetic and in the case of α -adrenoceptor mediated effects also to sympathomimetic agents (2). Denervation supersensitivity described by Cannon and Rosenblueth (1949) is unspecific, but in the rat bladder no conclusions about the specificity of the supersensitivity can be made because of the presence of adrenergic as well as cholinergic nerve fibres in both the hypogastric and pelvic nerves (5).

The inhibitory β -adrenoceptor mediated effect on the bladder was not increased by any of the denervation procedures (2). This is in contrast to the excitatory (secretory) effect on the submaxillary gland of the rat which is also supplied with both α - and β -receptors and in which denervation supersensitivity develops both to α - and β -stimulating agents (Emmelin, Holmberg and Ohlin, 1965). It is, on the other hand, in accordance with the finding that denervation by section of the hypogastric or pelvic nerves to the cat urinary bladder does not result in greater irritability to the inhibitory action of adrenaline (Elliott, 1905).

The finding that a suprathreshold dose of a drug caused a larger response in the denervated than in the control bladder could partly be due to the hypertrophy of the detrusor muscle after parasympathetic denervation, but the response increased also after sympathetic denervation when no hypertrophy was found. Furthermore, the threshold doses to the drugs were lowered in accordance with the characteristics of a denervation supersensitivity (2).

CHAPTER IV

NERVE STIMULATION

Cholinergic fibres

The response of the rat urinary bladder to electrical stimulation of the pelvic nerves bilaterally was a contraction of the detrusor muscle increasing the intravesical pressure by about 60 mm Hg at a stimulation frequency of 15 Hz (6). This is in agreement with earlier findings (Carpenter and Rubin, 1967). The response to stimulation of the pelvic nerve distal to the pelvic ganglion was not affected by previous injection of the ganglionic blocking agent hexamethonium, indicating that the nerves stimulated were postganglionic (5). When the pelvic nerve was stimulated proximal to the pelvic ganglion the response was not totally abolished by hexamethonium, suggesting that some of the axons in the pelvic nerve originate from ganglion cells proximal to the pelvic ganglion, in agreement with the histochemical findings described in chapter III.

Electrical stimulation of the hypogastric nerves caused contraction of the detrusor muscle, increasing the intravesical pressure by about 5 mm Hg at 15 Hz (5). Vanov (1965) did not obtain any effect of hypogastric nerve stimulation when recording the response of the rat bladder as contractions in the axis from the urethra to the vertex, instead of changes in the intravesical pressure. The response to hypogastric nerve stimulation was not affected by hexamethonium, indicating that the fibres stimulated were postganglionic (5).

The contractile response to stimulation of either the pelvic or the hypogastric nerves was unaffected by the adrenoceptor blocking agents dihydroergotamine or propranolol, only slightly reduced by the adrenergic neurone blocking drug guanethidine but was reduced by about 60 % after atropine or the parasympatholytic agent Hoechst 9980 (5). After injection of eserine the response to stimulation of one pelvic nerve was increased by 40—50 % and the response to hypogastric nerve stimulation was increased about 3 times, while the responses to bilateral pelvic nerve stimulation or to stimulation of all nerves simultaneously were not significantly changed

(5; 6). A reduction of the response to pelvic nerve stimulation after atropine in the rat has been described earlier (Vanov, 1965). An atropine sensitive portion of the response to transmural stimulation of the urinary bladder *in vitro* was found by Chesher (1970) in the rat, but not in the cat and guinea-pig.

In spite of this partial atropine resistance it is suggested that the nerve fibres stimulated at this frequency in both the pelvic and hypogastric nerves are cholinergic, which is further supported by the atropine sensitivity of degeneration activity described in chapter III and the atropine sensitivity found at stimulation of degenerating pelvic or hypogastric nerves to be discussed below. Carpenter (1963) found that the contractions of the rat bladder elicited by its postganglionic fibres were potentiated after eserine. Further evidence supporting the concept of a cholinergic nature of the motor nerves to the rat urinary bladder is that acetylcholine is released *in vitro* after postganglionic nerve stimulation (Carpenter and Rand, 1965; Chesher, 1967), and that the acetylcholine release is antagonized by botulinum toxin (Carpenter, 1967). Furthermore, the acetylcholine synthesizing enzyme choline acetyltransferase is present in the rat urinary bladder with an activity comparable to that of other organs innervated by cholinergic autonomic nerves (Ekström, 1975). In a recent report ATP failed to elicit a response in the rat urinary bladder *in vitro* speaking against a "purinergic" nature of the innervation (Clark and Mitchelson, 1974).

Evidence of a non-adrenergic mechanism involved in sympathetic nerve-bladder transmission has been described in the cat (Sigg and Sigg, 1964; De Sy, 1972; Sundin and Dahlström, 1973) and in the guinea-pig (Mantegazza and Naimzada, 1967). In these species the bladder wall contains intramural ganglia and atropine reduced the response only at such high doses that a ganglionic blocking effect (Feldberg and Vartiainen, 1935) can be expected.

The contractile response of the urinary bladder to electrical stimulation of the pelvic nerve at low stimulation frequencies (0.1—1 Hz) was found to be almost completely atropine resistant (4). This finding might be consistent with the theory proposed by Chesher (1970) that small amounts of acetylcholine released from the nerve ending act predominantly on atropine resistant receptors within the neuromuscular junction. When, however, a larger amount of acetylcholine is released or the destruction of the transmitter is prevented by eserine, acetylcholine may diffuse to atropine sensitive receptors located further away from the nerve ending. A contraction of similar size caused by stimulation at 15 Hz of a pelvic nerve 20—30 hours after postganglionic section was totally abolished by atropine or

Hoechst 9980. After injection of eserine all the responses were increased about 3 times, while they were not affected by the α -adrenoceptor blocking agent dihydroergotamine. The contraction of the detrusor muscle caused by stimulation of degenerating hypogastric nerves was also abolished by atropine or Hoechst 9980 (4).

It may be concluded that the transmitter activating the detrusor at stimulation of the pelvic nerve as well as the hypogastric nerves at these frequencies probably is acetylcholine. The degenerating nerve fibres were in these experiments stimulated at a time after denervation when the distance between nerve terminal and receptor may begin to increase and the acetylcholine content of the nerve to decrease, at least according to studies in somatomotor nerves (Reger, 1959; Lissák, Dempsey and Rosenbluth, 1939). The atropine sensitivity of the responses to stimulation of degenerating nerves may thus be consistent with the "proximity theory" of Dale and Gaddum, which was discussed in chapter III.

The interaction between cholinergic fibres from the different autonomic nerves to the bladder was studied by stimulation of the pelvic nerves unilaterally and bilaterally and by simultaneous stimulation of the pelvic and hypogastric nerves (6).

The size of the contraction caused by unilateral pelvic nerve stimulation amounted to about 60 % of the response to bilateral stimulation. There was a functional overlap between the right and the left pelvic nerve of about 20 % (6), which was also found by Carpenter and Rubin (1967).

No antagonism between the pelvic and hypogastric nerves was found in the rat bladder (6). The responses to stimulation of the pelvic and hypogastric nerves were instead added at simultaneous stimulation and in some rats this response was even bigger than the sum of the responses produced by the nerves stimulated separately (6). This might indicate a spatial summation in the effector cells innervated by cholinergic fibres from the pelvic and hypogastric nerves. This postulates a convergence by some of the fibres from the pelvic and hypogastric nerves. In the rat bladder cholinergic neuroterminals are in contact with every muscle cell (El-Badawi and Schenk, 1966).

Debaisieux (1912) also found synergistic contractions when stimulating the pelvic and hypogastric nerves simultaneously in the dog, cat and rabbit. The contractile response to hypogastric nerve stimulation in the cat is caused via adrenergic fibres (Edge, 1955; Edvardsen and Setekleiv, 1968). Edvardsen (1968) found no synergistic effects of concomitant pelvic and hypogastric nerve stimulation in the cat but only reduction of the detrusor contraction as compared to the response to stimulation of the pelvic or

hypogastric nerves separately. Sympathetic inhibition of intramural parasympathetic ganglia was suggested, since adrenergic nerve terminals forming synaptic structures around non-adrenergic cells have been demonstrated histochemically in the bladder wall of the cat (Hamberger and Norberg, 1965). The urinary bladder of the rat, in contrast to other species, contains no intramural ganglion cells (5).

Adrenergic fibres

Adrenergic as well as cholinergic nerve fibres innervating the rat urinary bladder were detected histochemically in both the pelvic and hypogastric nerves (5) and the presence of both excitatory α -adrenoceptors and inhibitory β -receptors in the detrusor muscle was shown (2). The bladder responses to stimulation of adrenergic fibres in the pelvic and hypogastric nerves were therefore studied (7). When the pelvic or hypogastric nerves were stimulated for 5 seconds the contraction of the detrusor was followed by relaxation in some rats. After the injection of atropine the contraction was reduced as described above and was always followed by relaxation when the nerves were stimulated for such a short period (7). When the stimulation period was longer the relaxation was probably masked by predominant contraction.

In dogs and cats some authors have found a relaxation of the bladder after an initial contraction when stimulating the hypogastric nerves (Griffiths, 1895; Kuntz and Saccomanno, 1944) and the pelvic nerves (Debaisieux, 1912). The contraction at hypogastric nerve stimulation in the cat is probably caused by activation of excitatory α -adrenoceptors and the relaxation by inhibitory β -receptors (Edvardsen, 1968). The relaxation is more protracted than the contractile response similar to the relaxation of the rat bladder found when the pelvic or hypogastric nerves were stimulated for a short period and the contractile response was reduced by atropine.

The relaxation of the detrusor muscle was abolished by guanethidine indicating that it was caused by stimulation of adrenergic fibres demonstrated histochemically in both the pelvic and the hypogastric nerves (5). It was shown in pharmacological experiments that the inhibitory β -adrenoceptors of the rat urinary bladder belong to the type of receptors classified as β_2 -receptors in other organs (3). The β_1 -receptor blocking compound practolol did not affect the inhibitory bladder response to nerve stimulation when given in a dose which completely blocks the β_1 -receptors of the heart in the cat (Åblad, Carlsson and Ek, 1973). The relaxation of the bladder was, however, totally abolished by the non-selec-

tive β -receptor blocking agent propranolol or the β_2 -blocking agent H ³⁵/₂₅, indicating that the inhibitory effect of adrenergic nerve stimulation is exerted by action on β_2 -receptors (7).

An increase of the contraction of the detrusor muscle was obtained when one of the pelvic nerves or the hypogastric nerves were stimulated at high frequencies (50–100 Hz). The response to bilateral stimulation of the pelvic nerves was not significantly changed by stimulation at these high frequencies (7). The increased part of the responses caused by the high stimulation frequencies was not affected by hexamethonium, atropine or propranolol but was abolished by guanethidine or dihydroergotamine, suggesting that postganglionic adrenergic fibres were stimulated acting on α -receptors. The presence of excitatory α -adrenoceptors in the rat detrusor muscle was shown pharmacologically (2). Garry and Gillespie (1955) found the same high stimulation frequency to be optimal for stimulation of the sympathetic nerves to the rabbit colon.

Hypothetically, the explanation of the finding that the α -receptors were not activated at low stimulation frequencies but only at these high frequencies might be an overflow of transmitter from the adrenergic fibres shown to be present in the detrusor muscle partly around blood vessels (5) activating α -receptors in muscle fibres not innervated by adrenergic nerves. The adrenergic innervation of the rat detrusor is sparse and the terminal fibres are not associated with individual muscle cells but only with large groups of muscle fibres (El-Badawi and Schenk, 1966; 5). The assumption that the excitatory α -receptors are activated when the amount of transmitter released is increased is in agreement with the results of Edge (1955), who obtained a bladder contraction in the cat by intraarterial injection of noradrenaline, but relaxation after equivalent doses intravenously. Two weeks after denervation of the rat bladder the response to injected noradrenaline was changed from predominant relaxation to contraction, probably because of sensitization of α -receptors more than β -receptors (2). Six to ten weeks after parasympathetic denervation an outgrowth of new adrenergic terminals in the detrusor muscle of the cat is observed changing the normal inhibitory β -receptor response to hypogastric nerve stimulation into an excitatory α -receptor response (Sundin and Dahlström, 1973). Thus existing but not utilized α -receptors might be supposed to be activated, postulating that these receptors demand a higher concentration of noradrenaline than do the β -receptors.

SUMMARY AND CONCLUSIONS

Summary

1. The autonomic innervation of the urinary bladder was studied in the male rat *in vivo* by means of injection of autonomic drugs, denervation experiments, histochemistry and nerve stimulation.

2. The parasympathomimetic drugs acetylcholine and methacholine caused contraction of the detrusor muscle which was totally abolished by previous injection of atropine. The sympathomimetic drugs adrenaline and phenylephrine elicited contraction, while isoprenaline caused relaxation. The response to noradrenaline varied but was most often relaxation. The presence of both excitatory α -adrenoceptors and inhibitory β -receptors in the detrusor muscle was shown, and the β -receptors were found to belong to the type of inhibitory receptors classified as β_2 -receptors in other organs.

3. During the second and third day after postganglionic parasympathetic denervation a degeneration activity was found, which was abolished by atropine or the parasympatholytic agent Hoechst 9980.

Two weeks after postganglionic sympathetic denervation no detectable change of adrenergic or acetylcholinesterase (AChE)-positive nerves was found at histochemical investigation, while parasympathetic decentralization or denervation produced a total disappearance of adrenergic nerves. The AChE-positive nerves were appreciably reduced in number after parasympathetic decentralization and not detectable after postganglionic denervation.

The bladder developed denervation supersensitivity to parasympathomimetic drugs and in the case of excitatory α -adrenoceptor mediated effects also to sympathomimetic drugs after parasympathetic decentralization and denervation and after sympathetic denervation. The inhibitory β -receptor effects, however, were not increased by any of the denervation procedures.

4. Electrical stimulation of the hypogastric nerves or the pelvic nerves distal to the pelvic ganglia elicited contraction of the detrusor muscle. The responses were not affected by the ganglionic blocking agent hexamethonium or the adrenoceptor blocking drugs dihydroergotamine and propranolol, only slightly reduced by the adrenergic neurone blocking drug guanethidine but were reduced by about 60 % after atropine and the atropine-like drug Hoechst 9980. Eserine potentiated the responses. Stimulation of the pelvic nerve proximal to the pelvic ganglion was partially blocked by hexamethonium.

The contractile response of the bladder to stimulation of degenerating pelvic or hypogastric nerves 20–30 hours after section of the nerves postganglionically was abolished by atropine or Hoechst 9980.

A functional overlap between the right and the left pelvic nerve of about 20 % was found, while the contractile response to stimulation of the hypogastric nerves was added to the pelvic nerve response with no functional overlap or antagonism.

The contraction of the detrusor muscle caused by stimulation of the pelvic or hypogastric nerves was followed by relaxation when the nerves were stimulated for a short period and the initial contraction was reduced by atropine. The relaxation was found to be mediated by adrenergic fibres acting on inhibitory β_2 -receptors. Stimulation of the pelvic or hypogastric nerves at high frequencies increased the contractile response probably *via* adrenergic fibres activating excitatory α -receptors.

Conclusions

From the results of the experiments described in this thesis it may be concluded

that the detrusor muscle of the rat is activated by cholinergic fibres when the parasympathetic pelvic nerves or the sympathetic hypogastric nerves are stimulated at physiological frequencies,

that the contractile responses to stimulation of cholinergic fibres in the pelvic and hypogastric nerves are added at simultaneous stimulation with no functional overlap or antagonism,

that the detrusor receives postganglionic adrenergic as well as postganglionic cholinergic nerve fibres from both the pelvic and hypogastric nerves (see Figure),

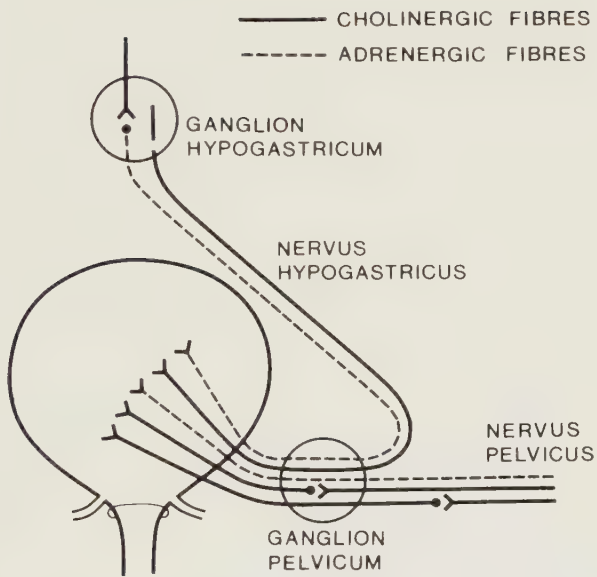
that the detrusor muscle contains both excitatory α -adrenoceptors and inhibitory β_2 -receptors and

that these receptors can be activated *via* the vesical nerves using high stimulation frequencies or short stimulation periods, respectively.

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FIGURE



Proposed arrangement of the autonomic innervation of the rat detrusor muscle. The nerves of one side are shown.

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